Cellular softening mediates leukocyte demargination and trafficking, thereby increasing clinical blood counts

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Leukocytes normally marginate toward the vascular wall in large vessels and within the microvasculature. Reversal of this process, leukocyte demargination, leads to substantial increases in the clinical white blood cell and granulocyte count and is a well-documented effect of glucocorticoid and catecholamine hormones, although the underlying mechanisms remain unclear. Here we show that alterations in granulocyte mechanical properties are the driving force behind glucocorticoid- and catecholamine-induced demargination. First, we found that the proportions of granulocytes from healthy human subjects that traversed and demarginated from microfluidic models of capillary beds and veins, respectively, increased after the subjects ingested glucocorticoids. Also, we show that glucocorticoid and catecholamine exposure reorganizes cellular cortical actin, significantly reducing granulocyte stiffness, as measured with atomic force microscopy. Furthermore, using simple kinetic theory computational modeling, we found that this reduction in stiffness alone is sufficient to cause granulocyte demargination. Taken together, our findings reveal a biomechanical answer to an old hematologic question regarding how glucocorticoids and catecholamines cause leukocyte demargination. In addition, in a broader sense, we have discovered a temporally and energetically efficient mechanism in which the innate immune system can simply alter leukocyte stiffness to fine tune margination/demargination and therefore leukocyte trafficking in general. These observations have broad clinically relevant implications for the inflammatory process overall as well as hematopoietic stem cell mobilization and homing.

cellular mechanics | leukocyte deformability | demargination | microfluidics | atomic force microscopy

Leukocyte margination within the microvasculature and in larger blood vessels is an integral part of the inflammatory process and innate immune system (1, 2). This margination phenomenon is twofold, involving sequestration of leukocytes in the capillary bed (3, 4) as well as movement of leukocytes toward the blood vessel wall (Fig. 1*A*) (5, 6). Recent experimental and computational data, including our own, indicate that the mechanical properties of leukocytes play a major role in margination and are sufficient to drive leukocytes in whole blood toward the vessel wall (7–12).

What is not known is whether leukocyte softening can cause the reversal of leukocyte margination, which would indicate that leukocyte stiffness may be modulated by the immune system as an additional biophysical means to mediate leukocyte trafficking. To that end, we explored whether leukocyte stiffness alterations play a role in leukocyte demargination induced by glucocorticoid and catecholamine hormones. Although this phenomenon, which causes significant increases in the white blood cell (WBC) count within the clinical complete blood count (CBC) and specifically involves the granulocyte subpopulation of leukocytes, has been well documented from a clinical perspective for decades, the underlying mechanisms remain unclear (13–15). This leukocyte demargination effect can be induced via in vivo ingestion of an exogenous glucocorticoid, such as dexamethasone, or catecholamine, such as epinephrine, both of which are used clinically as an antiinflammatory agent and a vasopressor, respectively. Canonically, glucocorticoid- and catecholamine-induced demargination is attributed to down-regulation of adhesion molecules such as L- and P-selectin (16). However, in humans, although glucocorticoid infusion is associated with decreased leukocyte L-selectin expression, this does not occur until several hours after leukocyte demargination and concurrent increase in leukocyte count has already transpired (17). Furthermore, L- and P-selectin-deficient mice exhibit no abnormalities in leukocyte margination compared with wild type, suggesting additional mechanisms are likely involved (18, 19). Finally, leukocyte margination toward the vessel wall occurs in vitro in the absence of intact endothelium, questioning the need for specific interactions between these ligands and their adhesion molecules during this process (5, 6).

Mechanistically, computational models have determined that cell–cell collisions between leukocytes and softer erythrocytes

Significance

Clinical hematologists have long known that antiinflammatory glucocorticoids such as dexamethasone and blood pressure-supporting catecholamines such as epinephrine cause leukocytes to demarginate from the vascular wall and microvasculature into the main circulation, significantly elevating the effective white blood cell count. Canonically, this has been attributed to down-regulation of adhesion molecules such as selectins, but we show that a purely mechanical phenomenon caused by leukocyte softening plays a major role as well. Our work provides an answer to an old hematological problem and reveals a mechanism in which the immune system simply alters leukocyte stiffness to regulate leukocyte trafficking. This has clinically relevant implications for the inflammatory process overall as well as for hematopoietic stem cell mobilization and homing.

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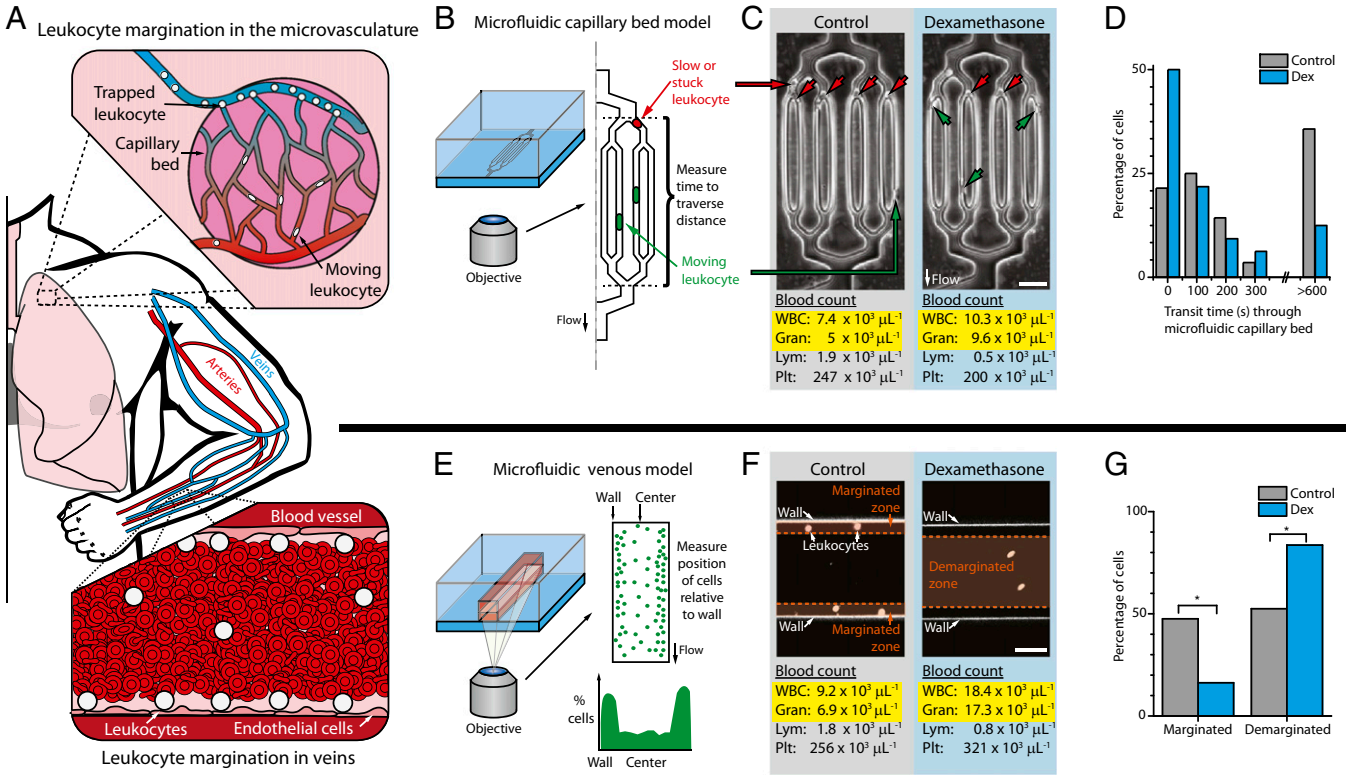

Fig. 1. A reductionist microfluidic approach to investigate whether alterations in the mechanical properties of leukocytes can cause demargination in the microvasculature and larger blood vessels. (*A*) Under homeostatic conditions, leukocytes marginate via two main mechanisms: sequestration within the microvascular capillary beds and movement toward the vessel wall in veins. (*B*) Transit times of leukocytes through a microfluidic capillary bed model were measured to determine the degree of margination within the microvasculature. (*C*) CBC performed on blood samples obtained from one healthy human subject before and after ingestion of dexamethasone (Dex) showed an expected increase in the WBC and granulocyte (Gran) counts. (*D*) Leukocytes isolated from the same subject after dexamethasone ingestion have shorter transit times than pretreatment control leukocytes obtained from the same subject, with less obstructed leukocytes within the microfluidic device ($P < 0.05$ via Mann–Whitney test). (*E*) Using confocal videomicroscopy and a microfluidic large veins model, the distances of leukocytes from the wall of the microchannel were measured to determine degree of margination. (*F*) CBCs performed on blood samples obtained from a second healthy human subject also showed the expected WBC and Gran increase after dexamethasone ingestion. (*G*) Leukocytes within whole-blood samples collected after dexamethasone ingestion demarginated away from the vessel wall in higher proportions compared with pretreatment controls ($N_{control} = 4{,}894$ cells, $N_{dex6hour} = 4{,}398$ cells, $P < 0.01$ via chi-square test), which correlates to the dexamethasone-induced increases in WBC and granulocyte counts.

enhance leukocyte margination (7, 8, 20, 21). Computational research from our own group and others indicate that modulating only the cell mechanical properties, such as stiffness, alters these physical interactions and thus changes in margination, but this has not been validated experimentally (9–12, 20–22). Furthermore, decreases in cell stiffness may also reduce leukocyte sequestration within the capillary bed, as softer cells could deform to release into the circulation. Here we demonstrate that granulocyte softening is the driving force behind glucorticoid- and catecholamine-induced demargination, including leukocyte release both out of the capillary bed and away from the vascular wall of larger vessels, and provides a cellular mechanical mechanism in which the mechanical properties of leukocytes directly contribute to increases in the WBC and granulocyte counts observed clinically. In addition, our findings reveal a biophysical answer to an old hematologic question regarding how glucocorticoids and catecholamines cause leukocyte demargination.

Results

Dexamethasone Ingestion in Human Subjects Leads to Increased Clinical Leukocyte Counts and Ex Vivo Leukocyte Demargination. Determination of whether physical mechanisms mediate leukocyte demargination requires an experimental system that enables tight control of biofluidic parameters but is devoid of the confounding factors found in vivo such as adhesive endothelial interactions, inflammatory mediators, bone marrow leukocyte release, and channel geometry changes. Accordingly, we used a reductionist approach and used two separate in vitro microfluidic systems. First, we used a microfluidic model of the microvasculature consisting of multiple capillary-sized channels 5. 9 ± 0.08 µm wide, which are smaller than the average leukocyte diameter of ~7.25 µm, to investigate whether glucocorticoid ingestion leads to changes in the mechanical properties of leukocytes that, in turn, would alter the degree of microvascular leukocyte sequestration (Fig. 1*B* and Fig. S1) (23). Six hours after dexamethasone ingestion by a healthy human subject, which led to the expected corresponding increase in leukocyte and granulocyte counts within the CBC (Fig. 1*C*), we found that isolated granulocytes from the subject traversed the microfluidic channels with significantly faster transit times and with a lower proportion of cells becoming stuck within the device compared with granulocytes obtained from the same individual before glucocorticoid ingestion (Fig. 1*D*). Microchannels are biochemically blocked to prevent nonspecific adhesion, so changes in transit time are due only to alterations in deformability rather than adhesion molecule down-regulation. As such, the reduction in granulocyte entrapment and transit time seen is a reflection of how leukocytes within the marginated microvascular pool in vivo are released into the circulation in response to glucocorticoid ingestion, thereby increasing the clinical WBC count.

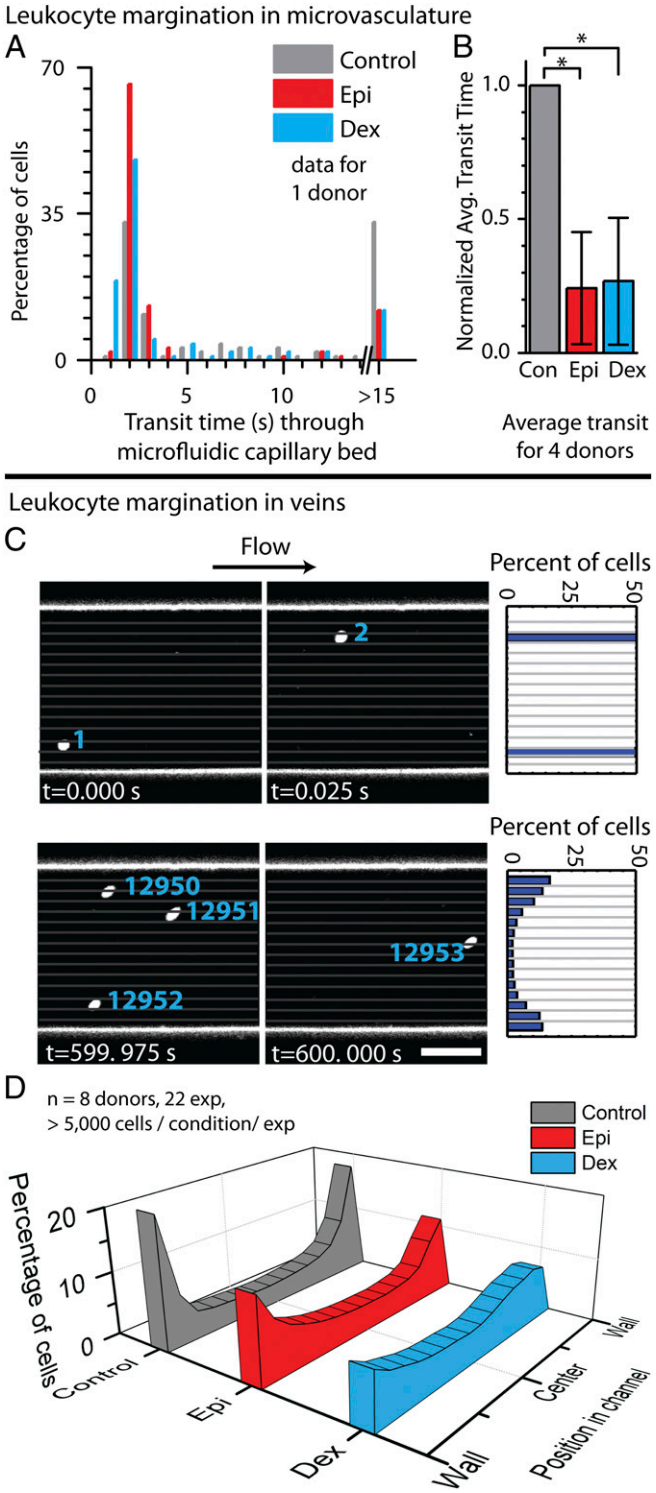

Fig. 2. In vitro exposure to glucocorticoids or catecholamines alter granulocyte trafficking in microfluidic systems. (*A*) Compared with untreated controls, isolated leukocytes from one representative healthy donor treated with the glucocorticoid dexamethasone (Dex) or the catecholamine epinephrine (Epi) have shorter transit times with less propensity to cause microchannel obstruction within the microfluidic capillary bed model ($P < 1 \times 10^{-5}$ for each condition via Mann–Whitney test). (*B*) Aggregate results from four donors further show that leukocytes exposed to dexamethasone or epinephrine have shorter transit times than control leukocytes ($P < 1 \times 10^{-6}$ via Mann–Whitney test). (*C*) Leukocyte demargination within the microfluidic venous model is tracked by imaging stained leukocytes within whole blood in microfluidics via confocal videomicroscopy. The leukocyte position with respect to the microfluidic wall was calculated using a custom MATLAB imaging processing script. (*D*) The histogram shows leukocyte locations (perpendicular to flow) pooled from eight donors over 22 experiments, with >5,000 cells analyzed per condition per experiment at physiological venous wall shear stress of 1 dyne/cm^2. Compared with untreated controls, leukocytes treated with dexamethasone strongly demarginated, and leukocytes treated with epinephrine showed a similar but less pronounced demargination.

Second, we used a microfluidic venous model consisting of a long, straight channel with a cross section of 150 μm by 150 μm to investigate whether glucocorticoid-mediated changes in leukocyte mechanical properties would alter the trafficking and positioning of leukocytes within whole blood with respect to the vessel wall (Fig. 1*E* and Fig. S1). With this approach, all changes in leukocyte position are the direct result of glucocorticoid or catecholamine effects on leukocytes rather than changes in endothelial adhesion molecules or bone marrow release. Whole blood with fluorescently stained leukocytes was perfused into the system, and individual leukocytes were tracked using confocal videomicroscopy at physiologic venous wall shear stresses (1 dyne/cm^2) (24). Six hours after dexamethasone ingestion by a healthy human subject, which again led to the expected corresponding increase in leukocyte and granulocyte count in the CBC (Fig. 1*F*), higher proportions of leukocytes were observed to travel away from the vessel wall toward the vessel center, the "demarginated zone" of the channel, compared with leukocytes tracked within whole blood samples obtained from the same subject before glucocorticoid ingestion (Fig. 1*G*). Taken together, the results from these two reductionist vascular microfluidic models show that the underlying mechanisms of how glucocorticoid ingestion in healthy human subjects increases the clinical WBC count and alters leukocyte trafficking in the microvasculature and larger vessels in a manner related to biophysical alterations of the leukocytes themselves and unrelated to the canonical explanation of adhesion molecule down-regulation.

In Vitro Treatment of Isolated Human Leukocytes with Glucocorticoids or Catecholamines Causes Demargination in Microfluidic Systems. To remove the potential confounding effects of pharmacokinetic variability among different subjects and to confirm that the observed changes in leukocyte trafficking are the direct result of the pharmacologic agents, we performed additional in vitro experiments using the microfluidic models with healthy human granulocytes and/or whole blood exposed, in vitro, to fixed concentrations of dexamethasone and epinephrine for fixed durations of time. Similar to our ex vivo experiments, the microfluidic capillary bed transit times of isolated granulocytes from one human subject directly treated with dexamethasone or epinephrine in vitro were significantly decreased compared with untreated control granulocytes, and a smaller proportion became trapped within the device (Fig. 2 *A* and *B*). We then conducted additional microfluidic venous experiments to quantify the degree to which leukocytes tend to marginate or demarginate under physiologic flow conditions, again using a combination of confocal microscopy and a custom-written image processing code (Fig. 2*C* and Movie S1). Our data reveal that simply incubating whole blood with dexamethasone or epinephrine causes in vitro leukocyte demargination in our microfluidic flow experiments (Fig. 2*D*). This dexamethasone- or epinephrine-induced leukocyte demargination persists with increasing levels of physiologic shear stress (Fig. S2). These experiments confirm that the effects seen from our ex vivo experiments described in Fig. 1 are directly the result of drug treatment and suggest that leukocyte demargination and sequestration may involve processes related to changes in the mechanical properties of the leukocytes and are external to adhesion molecule down-regulation. In addition, we observed that glucocorticoid and catecholamine-mediated leukocyte demargination

occurs in more physiologic microfluidic devices cultured with a confluent endothelial monolayer that spans the entire inner surface area of the device, providing further evidence that this demargination effect likely also occurs in vivo (Fig. S3) (25).

To confirm that the observed demargination phenomenon is due to dexamethasone- or epinephrine-induced changes only to leukocytes and not erythrocytes, erythrocytes or granulocytes were isolated and individually exposed to dexamethasone or epinephrine, recombined to physiologically relevant levels, and examined for margination or demargination. No significant changes in demargination were observed in mixtures of untreated granulocytes and treated erythrocytes compared with an untreated control suspension, whereas changes in demargination comparable to a treated suspension were observed in mixtures of treated granulocytes and untreated erythrocytes (Fig. S4).

Glucocorticoids and Catecholamines Reduce the Mechanical Stiffness of Human Granulocytes via Cytoskeletal Remodeling. As alterations in the mechanical properties of the leukocytes would lead to the faster transit times seen in the microfluidic capillary model after drug treatment, we hypothesized that glucocorticoids and catecholamines decrease leukocyte stiffness. Atomic force microscopy (AFM) measurements (Fig. 3*A*) confirmed that epinephrine- and dexamethasone-treated granulocytes were significantly softer than time-controlled untreated granulocytes ($P < 1 \times 10^{-4}$ and 1×10^{-3}, respectively). These degrees of reduction in granulocyte stiffness corresponded to the degree of reduction in transit times in the microfluidic capillary bed model and the degree of demargination observed in the microfluidic venous model for both dexamethasone and epinephrine treatment.

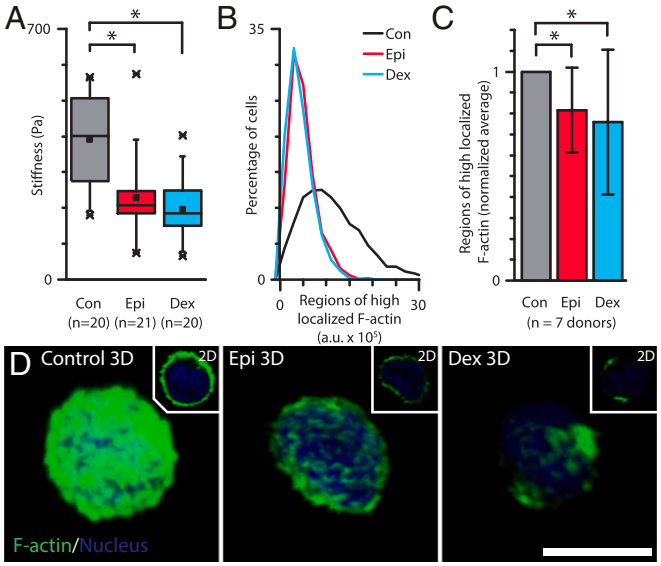

Fig. 3. Granulocytes exposed to glucocorticoids and catecholamines are mechanically softer due to reorganization of the cortical actin cytoskeleton. (*A*) AFM measurements of granulocytes from a single donor exposed to physiologically relevant doses of glucocorticoids (Dex) and catecholamine (Epi) reveal a significant reduction in cellular mechanical stiffness compared with untreated controls ($P < 0.0001$ and 0.001, respectively.) Box plot denotes 25th and 75th percentile; "x" denotes first and 99th percentile, with central dot representing the mean value. (*B*) Flow cytometry analysis of phalloidin staining reveals significant reductions in localized, polymerized actin in granulocytes exposed to dexamethasone and epinephrine for one representative donor. (*C*) Aggregate flow cytometry results from six donors further show reduced localized actin in treated leukocytes compared with untreated leukocytes ($P < 0.01$ via Mann–Whitney test.) (*D*) Confocal microscopy images show less polymerized actin in granulocytes after exposure to epinephrine and dexamethasone. (Scale bar, 5 μm.)

Flow cytometry (Fig. 3 *B* and *C*) and confocal microscopy (Fig. 3*D*) revealed that exposure to dexamethasone and epinephrine reorganize the granulocyte actin cytoskeleton, providing a mechanism for the observed reductions in cell stiffness. A bright, continuous cortical polymerized actin ring and high amounts of localized actin were observed in untreated control granulocytes. In contrast, epinephrine-treated granulocytes revealed a dimmer, but still continuous, polymerized actin ring, and a highly heterogeneous, discontinuous cortical polymerized actin ring was observed in dexamethasone-treated granulocytes (Movie S2). These differential degrees of cortical actin and localized polymerization in epinephrine- and dexamethasone-exposed granulocytes corresponded with the degrees of leukocyte demargination seen in our microfluidic experiments. In addition, no change in the granulocyte size was observed with epinephrine or dexamethasone exposure (Fig. S5). The architecture of intermediate filaments (vimentin), a key part of the leukocyte cytoskeleton, revealed little change after leukocyte exposure to dexamethasone or epinephrine (Fig. S6). Previous studies in other cell types have shown that activation of glucocorticoid receptors and adrenergic receptors (i.e., catecholamine receptors) leads to downstream rearrangements of cytoskeletal actin via multiple pathways including SGK1, ERK, RhoA/ROCK1, and cAMP-dependent protein kinase A signaling (26–31), providing candidate pathways that could provide further insight into our observed phenomenon. Of note, erythrocyte exposure to dexamethasone or epinephrine revealed no measurable changes in red cell concentration (i.e., no aggregation), size, shape, deformability, or margination (Figs. S6 and S7 and Table S1).

Mathematical Models Confirm That Leukocyte Mechanical Properties Regulate Margination or Demargination in Large Vessels. Although reduced mechanical stiffness explains fast transit through a microfluidic capillary bed, the link between cellular stiffness and demargination in larger veins is less clear. Performing a direct cell-level simulation of whole blood with leukocytes of various stiffnesses in a 150-μm channel is computationally infeasible. Nor is it necessary, because the key issue at hand is simply the role of leukocyte flexibility in margination, with all other parameters held constant. To address this issue, we observe that the primary mechanisms for cell transport relative to the vessel walls in blood are (*i*) collisions between cells as the flow drives them past one another (Fig. 4*A*) and (*ii*) movement away from walls due to induced secondary flow around the cells (Fig. 4*B*). When a stiff cell (i.e., a leukocyte) interacts with a soft cell (i.e., an erythrocyte), the soft one deforms, whereas the stiff one, which does not deform, will displace further from the site of collision (Fig. 4*A*) (9). Over the course of multiple collisions, stiff cells will reach the vessel wall. Due to the shear near the wall, a softer cell near the wall deforms and orients toward the flow direction more than a stiff one. This cell "pulls back" on the surrounding fluid, generating a secondary flow that drives fluid toward the wall (Fig. 4*B*). However, as fluid cannot pass through the wall, a lift force on the cell is generated, and, in response, it moves away from the wall. [In theoretical terms, the deformed cell can be idealized as a force dipole; the image of this dipole induced by the presence of the wall generates a flow that drives the cell away from the wall (32).] As a cell becomes softer, this effect becomes more pronounced.

We now describe two sets of results that integrate these mechanisms and illustrate the qualitative effect of leukocyte stiffness changes on margination. First, we used multiphase flow simulations and model blood as a suspension of two populations of deformable particles, one stiffer (which represents leukocytes) and one softer (which represents erythrocytes), flowing in simple shear between parallel walls. The simulations use a kinetic Monte Carlo method (10, 11). The results show that modulating the relative stiffness between leukocyte and erythrocytes determines the degree of leukocyte margination (Fig. 4*C*). Although these simulations do not take into account the size and shape differences between cells, due to

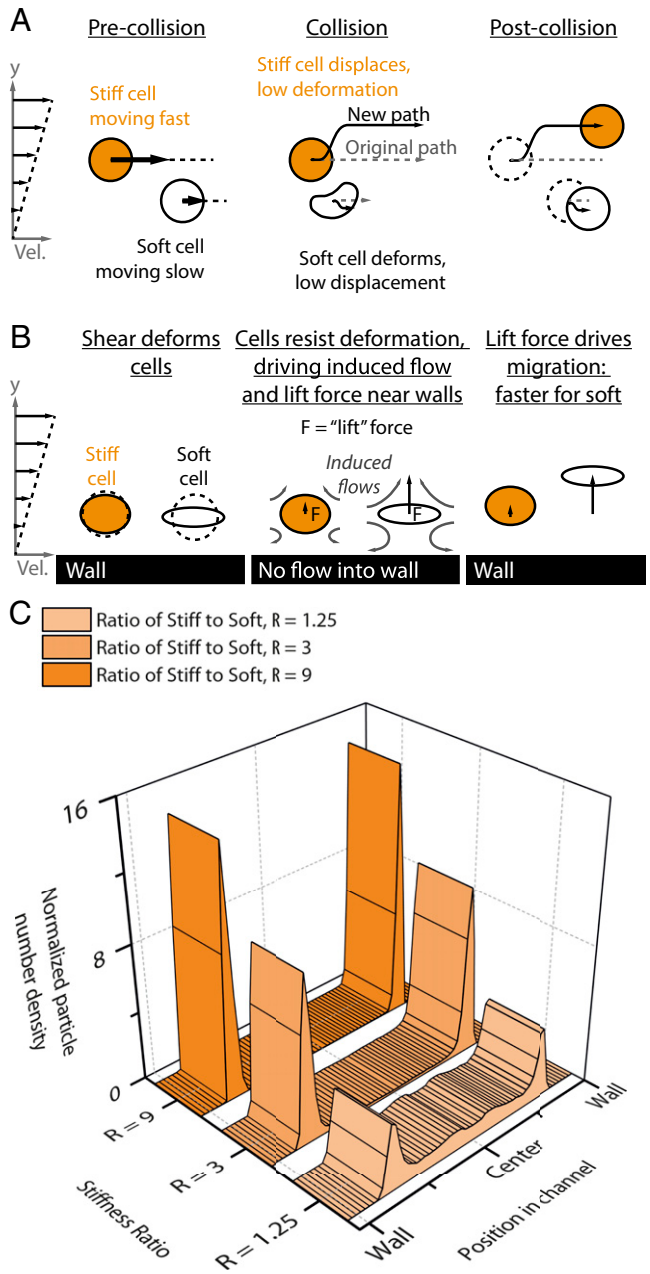

Fig. 4. Computational modeling of deformable particles reveals that modulating particle stiffness alone is sufficient to cause margination or demargination. (*A*) The first mediator of particle margination is cross-stream diffusion due to particle–particle collisions in which stiff particles displace whereas soft particles deform but do not displace. (*B*) The second mediator is a variable lift force near the wall that is dependent on particle stiffness. (*C*) Simulations reveal that, under typical conditions, stiff particles (leukocytes) marginate toward the vessel wall in the presence of a dominant softer component (erythrocytes). As the stiff particles (leukocytes) soften, demargination occurs, creating a more uniform distribution across the channel. Here, R is the stiffness ratio between stiff particles (leukocytes) and soft particles (erythrocytes).

computational complexity, the overall demargination effect as leukocyte stiffness is decreased should remain relatively unchanged (12).

Finally, these results as well as the experiments are consistent with a recent analytical theory that can be derived as a limiting case from the model used above (12). This theory shows that the tendency toward margination is controlled by a "margination parameter" M. This quantity scales as follows: $M \propto \rho_m - \rho_c$, where ρ_m and ρ_c are the following ratios:

$$\rho_m = \frac{migration\ velocity\ of\ WBC}{migration\ velocity\ of\ RBC};$$
$$\rho_c = \frac{collisional\ displacements\ of\ WBC}{collisional\ displacements\ of\ RBC}.$$

As M increases, the propensity to margination weakens. As the WBCs become softer, ρ_m increases and ρ_c decreases; both of these effects increase M, weakening margination in agreement with the experimental observations.

Discussion

In this work, we show that, upon exposure to glucocorticoids such as dexamethasone or catecholamines such epinephrine, the leukocyte cytoskeleton remodels and softens. As a result of this decrease in cell stiffness, previously trapped or slow-moving leukocytes in the capillary bed more rapidly pass through and enter the circulation (Fig. 5*A*). Concurrently, marginated leukocytes in the vessels soften, experience a larger lift force, and demarginate to a new equilibrium position farther away from the wall (Fig. 5*B*). These two phenomena, explained by reduced cellular mechanical stiffness, increase the clinical WBC and granulocyte counts, matching our ex vivo and in vitro experimental results with decades-old clinical observations relating leukocyte demargination as a side effect of treatment with glucocorticoids and catecholamines.

In addition, our findings not only reveal a biophysical solution to an old clinical "mystery" as to how glucocorticoids and catecholamines cause granulocyte demargination but also, in a broader sense, demonstrate that cellular mechanics principles are truly pervasive in human physiology and biomedicine. Specifically, ours is the first work, to our knowledge, to demonstrate that the mechanical properties of blood cells directly mediate the clinical CBC, the most commonly used biomarker/laboratory test in clinical medicine. Our data beg the question of whether other soluble

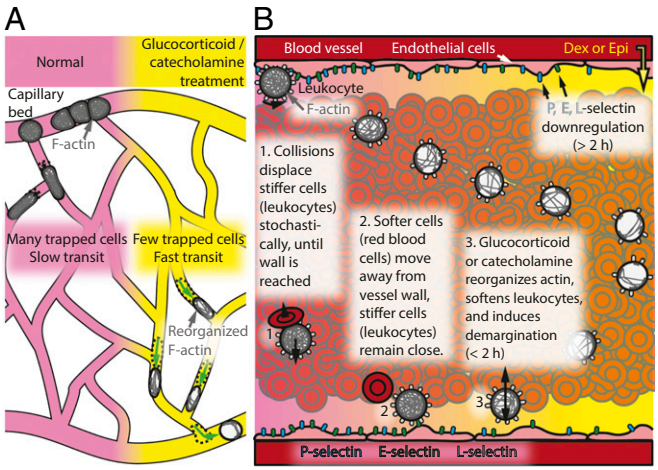

Fig. 5. Leukocyte softening causes demargination in the microvascular capillary beds and in larger blood vessels. (*A*) Glucocorticoids (such as dexamethasone) and catecholamines (such as epinephrine) induce actin reorganization, increasing leukocyte deformability. This leads to a decrease in the transit time through the capillary bed and reduces the number of sequestered cells. (*B*) Through a combination of collisions with softer erythrocytes and reduced wall demargination forces, stiffer leukocytes are normally predominantly marginated along vessel walls. Exposure to glucocorticoids or catecholamines subsequently leads to remodeling of the actin cytoskeleton, which softens the leukocytes, enabling them to demarginate and move toward the center, creating a more uniform distribution across the vessel.

factors, such as inflammatory mediators, would have similar effects on leukocyte margination. For example, the cytokines tumor necrosis factor (TNF)-α and interleukin (IL)-1β are both known to stiffen leukocytes (33, 34). Our results would suggest that, for this reason alone, these cytokines would cause biophysical alterations, in addition to the known biological effects, that lead to increased margination and increased endothelial interactions, which is consistent with the proinflammatory state of TNF-α and IL-1β upregulation. Noting the rapid time frames involved with the cellular stiffness changes we observed, our results also open up the possibility that changes in leukocyte stiffness may drive the initial steps of leukocyte recruitment during an inflammatory response that work in concert with changes in adhesion molecule expression and bone marrow release of additional leukocytes. Hence, we propose a complementary temporally and energetically efficient biophysical mechanism whereby the innate immune system can simply alter granulocyte stiffness to fine-tune margination/demargination and therefore leukocyte trafficking in general. This, in turn, would have broad clinically relevant implications for the inflammatory process overall and even hematopoietic stem cell mobilization and homing.

Methods

Sample Preparation. Blood was drawn according to institutional review board protocols approved by Georgia Institute of Technology into sodium citrate (Beckton Dickinson). Microfluidic capillary bed, AFM cell stiffness, flow cytometry, and single-cell imaging used granulocytes isolated with negative magnetic antibody-based selection (Miltenyi Biotec) or Percoll gradient (35) (Sigma-Aldrich). Samples were incubated with physiologically relevant concentrations of 10 μM dexamethasone for 2 h (36) or 50 pM epinephrine (Sigma-Aldrich) for 15 min (37). Incubation times coincide with onset of action for the drugs to cause leukocyte demargination (14, 15).

Microfluidic Experiments. Confocal microscopy tracked the position of acridine orange-stained leukocytes in whole blood perfused at physiological wall shear of 1 dyne/cm^2 through a 150-μm-tall by 150-μm-wide microchannel imaged 3 cm from the inlet of a 4-cm channel. A custom-written MATLAB code (MathWorks) extracted individual leukocyte positions from the series of confocal images. For endothelialized experiments, devices were endothelialized as previously described (25). For the capillary bed experiments, isolated granulocytes in PBS (1–3 × 10^6 cells per milliliter) were perfused through a microfluidic model of a capillary bed (23) at 0.250–1 μL/min. Cells in transit were recorded using brightfield microscopy and manually calculated.

AFM Cell Stiffness Experiments. Isolated granulocytes were attached to 0.01% poly-L-lysine (Sigma Aldrich)-treated glass AFM fluorodishes (World Precision Instruments). AFM calibration and methodology to measure cell stiffness were previously described (38).

Modeling of Soft and Stiff Cell Dynamics Within Vessels. Margination predictions were performed using a kinetic theory (master equation) description of cell dynamics (10) during simple shear flow between parallel walls and simulated using a kinetic Monte Carlo method (10, 11, 32, 39). Around 10^6 collisions were simulated at a hematocrit of 12%, with 1% of particles being stiff (WBCs) and the remainder soft (RBCs). Stiffer particles were up to 9 times stiffer than soft particles, and particles were of equal size. Additional details are given in *Supporting Information*.

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